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Screening for low grain cadmium phenotypes in sunflower, durum wheat and flax

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Summary

Cadmium (Cd) level in nonoilseed sunflower (*Helianthus annuus* L.), flax (*Linum usitatissimum* L.), and durum wheat (*Triticum turgidum* L. var. *durum*) grown on uncontaminated, alkaline soils has exceeded limits established in Northern Europe. Separate field experiments were conducted to investigate variability of grain Cd levels among sunflower, durum wheat and flax germplasm, and to seek an efficient screening method for future breeding. There were large variations in leaf Cd concentration among 200 sunflower lines. These lines performed more consistently for Cd uptake at the R5 stage than at the V8 stage across 4 locations with markedly differing soils. Cd concentration in V8 leaves was not related to Cd in grain. The positive correlation between R5 leaf Cd and kernel Cd level was obtained from nonoilseed hybrid (Sigco 954) (R^2 = 0.74**), and 200 lines (R^2 = 0.44**) tested over 4 locations in 2 field trials, respectively. This indicates that an efficient and low cost screening method can be developed for genotype selection, but plants must be grown to the R5 stage. A preliminary evaluation of 30 durum wheat and 74 flax lines indicated large variations in grain Cd level of durum wheat and flax. Grain Cd concentration ranged from 0.11 to 0.34 mg Cd kg^{-1} DW for durum wheat, and 0.14 to 1.37 mg Cd kg^{-1} DW for flax, respectively. This variability indicates that breeding for low grain Cd in durum wheat and flax should be feasible.

Abbreviations: DTPA – diethylenetriaminepentaacetic acid; DW – dry weight; FW – fresh weight

Introduction

Cadmium is a toxic element which naturally occurs in all soils (Holmgren et al., 1993). Current dietary intakes of Cd (10–15 μg day^{-1}) are below the FAO/WHO provisional tolerable weekly intake (70 μg day^{-1}) and do not constitute a health risk (Kowal et al., 1979; Adams, 1991). However, under some conditions where individuals consume only foods grown on Cd contaminated soils, Cd can accumulate in human kidney cortex and cause renal tubular dysfunction (Nogawa et al., 1987; Strehlow & Barltrop, 1988; McKenna and Chaney, 1991; International Programme & Chemical Safety, 1992).

International marketing of grain to Northern European nations requires meeting strict Cd limits (Figure 1). Plant species and cultivars vary genetically in the ability to absorb and translocate Cd to edible crop parts. Sunflower, durum wheat and flax are naturally higher in Cd than other grain crops. Cd in these crops grown on uncontaminated alkaline soil, exceeded German Guide Values (*Richtwert*) for some cultivars and soil series (Wolnik et al, 1983, 1985; Klein & Weigert, 1987; Ocker et al., 1991; Marquard et al., 1992; Chaney et al., 1993). The US and several other nations have conducted research to characterize soil series which could reliably produce grain with low enough Cd levels for shipment to Northern Europe. Recent research has shown that soil properties play an important role

in Cd uptaken and accumulation in grain crops (Eriksson, 1990; Chaney et al., 1993; Li et al., 1994; Tiller et al., 1995). Identification of soil series that produce low grain Cd in crops can be an effective method to lower grain Cd level. However, if the industry only purchases grain produced from certain soil types, a large acreage of traditional grain production fields might be limited. Therefore, efforts to breed widely adapted genotype(s) with low grain Cd accumulation characteristics across different soil series and environments are needed.

In a recent paper from our research group (Li et al., 1995a), it was shown that clear differences in kernel Cd level among 200 sunflower genotypes would allow breeding for lower kernel Cd concentration. We also conducted an experiment to assess the importance of combining ability and heterosis in the inheritance of sunflower kernel Cd levels in F_1 hybrids (Li et al., 1995b). Additive genetic effects predominantly influenced the expression of kernel Cd accumulation in hybrids. Others have reported genetic differences in grain Cd of corn and wheat. Hinesly et al. (1978) evaluated Cd uptake by 20 corn inbreds on long term sludge amended soils in the field; grain Cd ranged from 0.05 to 1.81 mg Cd kg^{-1} DW on one high Cd test soil. Andersson and Pettersson (1981) reported that the wheat cultivar 'Holme' had consistently lower grain Cd than four other wheat cultivars grown at five locations in Sweden.

Understanding the inheritance of Cd accumulation is important for breeding low Cd grain crops. Estimates of genetic variation and effects provide useful guidelines to determine the value of some populations and appropriate procedures to use in a breeding program (Li & Gabelman, 1990). Although there have been some efforts to study genotypic differences for Cd concentration in grain crops, little effort has been made to breed for lower Cd accumulation in products of agricultural plants. One barrier to this approach is the lack of an efficient screening method for Cd accumulation. Another is our lack of understanding of plant responses associated with Cd accumulation that might be useful as markers in a screening program (Wagner, 1993). Cultivar evaluation for grain Cd level is needed for both existing and new commercial cultivars, but analysis of grain Cd is quite expensive.

In order to comply with strict Cd standards which have been imposed for imported grain by several countries, a concerted effort is underway to develop a means of lowering the Cd concentration in the grain of nonoilseed sunflower, durum wheat and food flax (US), durum wheat and flax (Canada), and wheat (*Triticum aestivum* L.) and potato (*Solanum tuberosum* L.) (Australia). The objective of this study was to seek an efficient screening method for Cd accumulation in grain crops, and to evaluate variability of Cd concentration in sunflower, durum wheat and flax germplasm in order to developing successful breeding programs for low grain Cd cultivars.

Materials and methods

Plant sample collection

Two hundred sunflower genotypes, including inbreds, Plant Introduction lines and interspecific germplasm lines, were grown at four locations in North Dakota and Minnesota. Soil types at each location were described previously (Li et al., 1995a). The experiment was arranged in a randomized complete block design with 2 replicates at each location. The plots were single rows, 6.1 m long with 0.76 m between rows. Plants were thinned at the V4 stage; seedling leaf (V8 seedling stage) and diagnostic leaf (R5 anthesis stage) samples were collected (Schneiter & Miller, 1981). Each genotype was represented by 12 randomly selected plants in each replication. Kernel samples were obtained by non-Cd-contaminating threshing and dehulling procedures described previously (Li et al., 1995a). Commercial nonoilseed hybrid (Sigco 954) kernels and diagnostic leaves were sampled from four locations in North Dakota and Minnesota.

Thirty durum wheat lines were grown in a randomized complete block experiment with 4 reps (Uniform Regional Durum Nursery) at Langdon, North Dakota, on a Barnes fine loamy (Mixed Udic Hablobolls) soil. Within each replicate, plots consisted of four rows, 2.6 m long and spaced 0.76 m apart. Wheat grain was harvested and threshed at maturity.

Fourteen commercial flax cultivars and 60 Plant Introduction lines were grown in several plots on a Fargo silty clay soil (Vertic Haplaquolls). Flax grain samples were harvested and threshed at maturity.

Wheat and flax grain was threshed and processed by hand, or using devices without Cd and Zn surfaces in order to prevent Cd contamination of grain samples.

Analytic procedure

Grain (4 g per sample) and leaves (2 g per sample) were dry ashed in a muffle furnace at 480 °C for 16 h. Ash was digested with 2 ml of concentrated HNO_3,

and heated to incipient dryness; 10 ml 3N HCl was added, the beakers heated to reflux for 2 h, and then the solution was filtered into 25 ml volumetric flasks. Subsequently, Cd concentration was determined by flame atomic absorption spectrophotometry with deuterium background correction; an expanded scale was used. There were 10% duplicated samples in each batch for analysis of sunflower V8 and R5 leaves. Analyses of grain samples were conducted in duplicate. If duplicates differed by more than 0.25 fold, the sample was re-analyzed.

Ground sunflower kernel analytical reference standards and sample blanks were analyzed with each batch of samples. As a further quality assurance measure, about 15% of all samples were randomly selected for re-analysis, and showed good agreement with the original duplicate analyses.

Statistical analysis

Data was analyzed using SAS version 6.0 for personal computers (SAS Institute, 1989). The General Linear Model (GLM) procedure was used for analyses of variance over locations and replicates, and testing for genotype differences, location effects and genotype × location (G × L) interactions for sunflower V8 and R5 leaves. Flax lines were grown in only one replication in different plots on a Fargo silty clay soil. Therefore, geometric mean of seed Cd was carried out based on analytical replications. GLM was used to test genotype differences for Cd concentration in durum wheat and flax lines. Means were separated using the Waller-Duncan test after it was determined that there was a significant difference at 5% level for F value. Spearman rank correlation coefficients for sunflower leaf-Cd were determined among all locations. The relationships between sunflower kernel Cd content and V8 or R5 leaf Cd level were determined using SAS linear regression procedures.

Results and discussion

Separate field experiments were carried out to investigate low Cd lines of sunflower, flax and durum wheat. There were significant differences ($p < 0.01$) in grain Cd within each of the crop tested. These results indicate that low Cd genotypes can be selected for future breeding programs. The average Cd concentration was 1.51 mg kg^{-1} FW for 9 major nonoilseed sunflower hybrids (Li et al., 1995a), 1.21 mg kg^{-1} FW for 14 commercial flax cultivars (Table 1), and 0.228 mg kg^{-1} FW for 30 durum wheat lines (Table 2). These results are consistent with past research that has shown sunflower kernel Cd to be generally higher than flax and durum wheat grain Cd (Wolnik et al., 1983; Klein & Weigert, 1987; Ocker et al., 1991). In our series, sunflower and flax were grown on Fargo soils while the durum wheat was grown on a Barnes soil at different site, so that direct comparison of flax and sunflower to durum may not be valid. A previous study showed higher grain Cd for a single sunflower cultivar grown on Fargo vs Barnes soil (unpublished data).

Table 1. Mean grain cadmium concentration of 14 commercial flax cultivars grown in different fields on Fargo silty clay soil at Fargo, North Dakota

Cultivar Name	Geometric mean	
	Grain Cd	Standard deviation
	mg kg^{-1} FW	
Prompt	1.55	0.087
Linton	1.46	0.048
Culbert	1.43	0.084
Flor	1.39	0.102
Norlin	1.39	0.088
Linott	1.33	0.091
Clark	1.28	0.082
Neche	1.22	0.070
Verne	1.20	0.068
Norman	1.14	0.055
Day	1.08	0.072
McGregor	0.90	0.062
Dufferin	0.87	0.024
Omega	0.80	0.045

In general, comparison between these crops is problematic. Durum wheat is a cereal crop which has lower oil content than sunflower and flax grain. It is not known if there is any correlation between oil content and Cd concentration in grain crops. However, unlike sunflower and flax, peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.) kernels have lower Cd concentration and higher oil content than durum wheat (Figure 1). This may indicate that there is no relationship between the oil content of a crop and its patterns of Cd accumulation. The end use of each crop is also different. In general, a diet will contain higher levels of wheat grain than sunflower, so that the relative risk posed by increased Cd in sunflower may be lower due to lower consumption. Also, the factors involved in Cd uptake and translocation within each crop may be different. While several soil variables have been cor-

Table 2. Mean grain Cd concentration of 30 durum wheat lines grown on Barnes fine loamy soil with four replications at Langdon, North Dakota

Genotype	Grain Cd	Multiple comparison
	mg kg^{-1} FW	
Ward	0.343	a[1]
D87141	0.324	ab
Renville	0.310	ab
D87436	0.309	ab
D86-1523	0.305	abc
D86398	0.297	abcd
D87121	0.295	abcd
D88277	0.280	abcde
D88793	0.272	abcdef
D87130	0.272	abcdef
D88058	0.250	abcdefg
Rugby	0.240	abcdefgh
D87240	0.235	abcdefgh
D88758	0.234	abcdefgh
D88273	0.231	abcdefgh
D88303	0.230	abcdefgh
Munich	0.229	abcdefgh
Monroe	0.219	bcdefghi
D87122	0.213	bcdefghi
D87450	0.189	cdefghi
Sceptre	0.188	cdefghi
Lloyd	0.187	defghi
D88284	0.184	defghi
Vic	0.178	efghi
D88289	0.172	efghi
D86741	0.158	fghi
D88450	0.145	ghi
Mindum	0.137	ghi
D87-1534	0.125	hi
Medora	0.109	i

[1] Means followed by the same letter are not significantly different at the 5% level according to the Waller-Duncan K-ratio t-test.

related with Cd concentration in a variety of crops, it is not known if the variables play similar roles across crops (Tiller et al., 1995; Chaney et al., 1996).

Durum wheat contains much higher grain Cd concentration than non-durum wheat when grown on uncontaminated soils (Wolnik et al., 1983). Thirty durum wheat lines, representative of the germplasm available to breeders, were included in this study. Mean grain Cd concentration ranged from 0.11 to 0.34 mg Cd kg^{-1} FW (Table 2); all with values exceeding the German *Richtwert* (0.1 mg Cd kg^{-1} FW). Oliver et al. (1995) reported genetic variation for Cd concentration among Australian wheat cultivars, but cultivar effects were less significant than site (mainly soil properties) effects. In our study, soil DTPA-extractable Cd concentration of the experimental site ranged from 0.12 to 0.15 mg kg^{-1} among replicates indicating that the soil

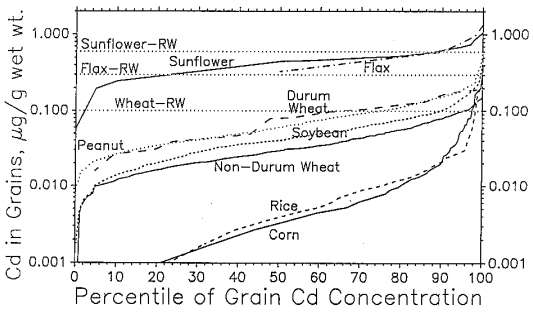

Figure 1. Statistical distribution of cadmium concentration in grain crops. Data for wheat, rice, corn, soybean, peanut from Wolnik et al. (1983, 1985); data for flax from Klein and Weigert (1987); data for sunflower from Ocker et al. (1991). German *Richtwert* (RW) for crops shown with horizontal dotted lines.

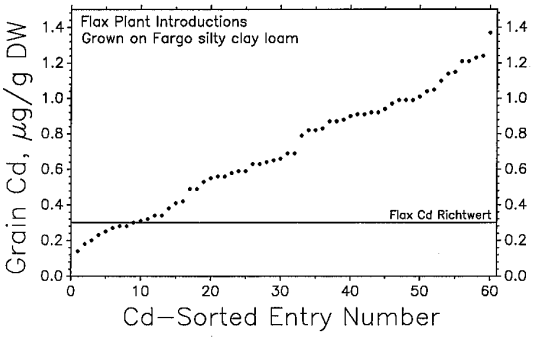

Figure 2. Distribution of grain cadmium concentration in different flax Plant Introduction lines grown at Fargo, North Dakota, on Fargo silty clay soil.

Table 3. Correlation (r) between locations for seedling leaf Cd concentration (top line), and diagnostic leaf Cd concentration (bottom line) of 200 sunflower genotypes grown at four locations in North Dakota and Minnesota

	Crookston	Grandin	Breckenridge
Fargo	0.30***	0.28***	0.19*
	0.50***	0.59***	0.46***
Crookston		0.21**	0.50***
		0.59***	0.44***
Grandin			0.27***
			0.45***

*, **, *** Significant at the 0.5, 0.01 and 0.001 probability level, respectively.

had not been contaminated with Cd by human activity. Soil pH was moderately low (5.8). Low soil pH can increase soil Cd solubility. In general, increases in soil Cd content result in increases in the uptake of Cd by plants (International Programme and Chemical Safety, 1992). The high phyto-availability of Cd in the soil may be an important factor causing the observed high Cd uptake in the durum lines evaluated.

In a previous study, we found that the fine textured Fargo soil had higher levels of DTPA-extractable Cd and total Cd than other soils tested. Sunflower grown on Fargo soil had significantly greater kernel Cd concentration across tested lines (Li et al., 1995a). Thus, Fargo silty clay soils at Fargo, ND, were chosen for a preliminary screening evaluation of Cd concentration in flax lines. Grain Cd concentration in 14 commercial flax cultivars, as shown in Table 1, exceeded the German *Richtwert* (0.3 mg Cd kg^{-1} FW) by 2.7 to 5.2 fold. Thus, low Cd characteristics should be incorporated in future commercial cultivars to allow production of low Cd grains in different soils. There were significant differences among 60 flax Plant Introduction lines for grain Cd concentration, with a range of 0.14 to 1.37 mg kg^{-1} FW (Figure 2). The average Cd concentration in the three lowest lines (0.17 mg kg^{-1} FW) was over seven fold lower than Cd level in the three highest lines (1.28 mg kg^{-1} FW). Marquard et al. (1990) reported that genotypic differences in uptake and accumulation of Cd exists among 16 flax genotypes grown at 5 locations in Germany. Their results also demonstrated that flax seed with low Cd content can be produced at suitable locations. The clear differences in grain Cd concentration among the 60 flax Plant Introduction lines tested indicates that low Cd line(s) can be selected for breeding programs. Further evaluation at different locations and years is necessary for parental line selection.

Previously we reported that genetic variation for sunflower kernel Cd was high. Mean kernel Cd concentration in four locations varied from 0.31 to 1.34 mg kg^{-1} FW across 200 sunflower lines (Li et al., 1995a). In addition to kernel Cd determinations, this experiment examined a variety of sunflower screening techniques. Determination of the genetic source of low Cd in grain crops is time consuming and costly. An efficient screening method is essential for a successful grain Cd breeding program. The analysis of grain requires that plants be grown to maturity, which is very labor intensive compared to leaf analysis. If leaf Cd and kernel Cd are correlated, a rapid screening method can be developed. To this end, sunflower leaf Cd levels at two growth stages (V8 stage: seedling leaf; R5 stage: diagnostic leaf) were compared to kernel Cd.

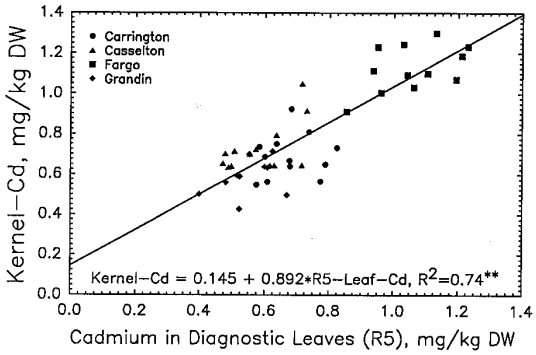

Figure 3. Regression of kernel Cd on seedling leaf Cd concentration of nonoilseed sunflower Hybrid 954 grown at four locations in North Dakota and Minnesota. ** Indicates significance of regression model at P = 0.01 level.

Large variations in seedling leaf Cd (V8) and diagnostic leaf Cd (R5) concentration were found among the 200 sunflower lines, respectively (data not shown). Across locations and lines, seedling leaf CD ranged from 0.13 to 2.47 mg kg^{-1} DW, and diagnostic leaf Cd concentrations were 0.26 to 2.67 mg kg^{-1} DW. Among tested lines, mean of 4 locations varied from 0.47 to 1.91 mg kg^{-1} DW for seedling leaf Cd, and 0.49 to 1.67 mg kg^{-1} DW for diagnostic leaf Cd. Higher Cd concentration for seedling leaf and diagnostic leaf was found at Fargo and Grandin than at the other two locations. Soils at Fargo and Grandin have lower soil pH values, higher levels of DTPA-extractable Cd and higher total soil Cd than soils at Crookston and Breckenridge (Li et al., 1995a). Soil properties influence Cd uptake and accumulation in grain crops and other plant species was also reported previously (Eriksson, 1990; Chaney et al., 1993; Li et al., 1994; Tiller et al., 1995). Rank correlation was used to compare the leaf Cd concentration of the tested lines among the locations. Correlation between locations for seedling leaf Cd and diagnostic leaf Cd were significant (Table 3). However, the r values were relatively low for seedling leaf Cd, ranging from 0.19 to 0.50. For diagnostic leaf Cd, the r values varied from 0.44 to 0.59. These results indicate that leaf Cd at the R5 stage is a better predictor of grain Cd than at the V8 stage across 4 locations with markedly different soils. Leaf Cd concentration of these 200 lines at either V8 or R5 stages, showed continuous variation across the range, indicating that leaf Cd is not simply inherited. These results for leaf Cd agree with results on genotypic variation in kernel

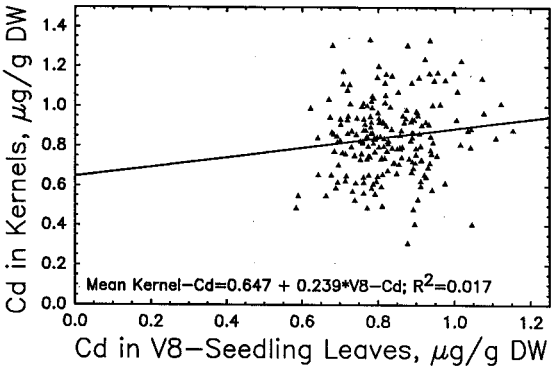

Figure 4. Regression of kernel Cd on seedling leaf (V8 leaf) Cd concentration of 200 sunflower genotypes grown at four locations in North Dakota and Minnesota. R^2 = 0.017 was not significant P = 0.05 level.

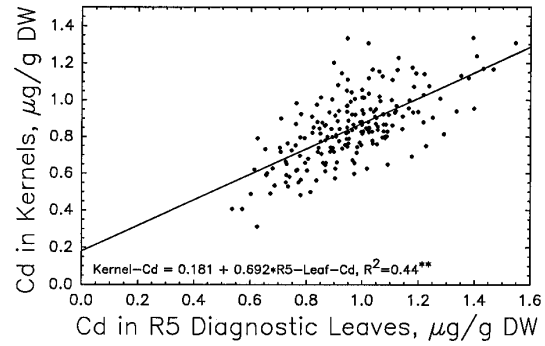

Figure 5. Regression of kernel Cd on diagnostic leaf (R5 leaf) Cd concentration of 200 sunflower genotypes grown at four locations in North Dakota and Minnesota. ** Indicates significance of regression model at P = 0.01 level.

Cd concentration in 200 sunflower genotypes (Li et al., 1995a).

In the study of one cultivar, commercial nonoilseed hybrid 954, kernels and diagnostic leaves were sampled from 4 locations in North Dakota and Minnesota. Regression analysis showed that the R square value was significant at the 1% level of probability over 4 locations indicating a positive relationship between kernel Cd and diagnostic leaf Cd concentration (Figure 3). In 200 lines, correlation between seedling leaves and harvested kernel Cd concentration was poor (Table 4 and Figure 4). This indicates that Cd uptake in the seedling stage is not a good indicator of kernel Cd concentration. This may be due to different genes controlling Cd uptake and accumulation. It may also be a function of plant rooting depth at the V8 stage are not as deep as at later stages. Further investigation of the rooting depth, soil moisture level and other factors affect Cd uptake is needed for elucidation of plant Cd level at different growth stages. At anthesis (R5), correlations between kernel Cd and leaf Cd level were significant at the 1% level at each location (Table 4). Regression analysis showed that R square was significant at the 0.1% level of probability over 4 locations (Figure 5). These results may indicate that Cd translocation and accumulation in sunflower leaf at anthesis stage and kernel are controlled by similar genetic factors. The positive relationship between diagnostic leaf Cd and kernel Cd could be used to eliminate some lines in a preliminary selection. Those lines that have no other superior desirable characteristics, yet still have high leaf Cd concentration could be discarded without great threat of losing potentially useful material. Since leaf analysis saves the time and expense, diagnostic leaf Cd may be a cost effective guide for rapid screening of germplasm and selection of low Cd genotypes in segregating populations in a breeding program.

Plant breeding selection for low Cd genotypes is nevertheless hindered by the high cost of laboratory analysis of leaves or grain to determine Cd concentrations. Another alternative screening method is to utilize genetic markers to assist in the selection of low Cd lines in grain crops. Penner et al. (1995) identified two RAPD markers linked to a gene governing Cd uptake in durum wheat. Of particular interest in this study was the observed correlation between diagnostic leaf Cd and kernel Cd. This may allow us to obtain genetic markers (e.g. RAPD and RLFP markers) which are linked with leaf Cd and kernel Cd using diagnostic leaf tissue. Probing for these markers on leaf DNA extracts would predict the kernel Cd level of different genotypes. This method would allow rapid screening of genotypes at a much lower cost than current techniques.

In CODEX ALIMENTARIUS (the WHO regulatory and advisory program for contaminants and food additives in international food shipments), several nations have been seeking a grain Cd limit of 0.10 mg kg^{-1}. This level is far lower than the limit needed to protect the health of persons who consume Cd in staple foods for 50 years (Chaney & Ryan, 1994; Nogawa et al., 1987). We believe that setting the Cd limit at 0.10 mg kg^{-1} is not needed to protect even the most exposed and the most sensitive persons for ingested Cd. The 0.10 mg Cd kg^{-1} grain level is exceeded by a fraction of the grain crops in most nations, but is exceeded by almost all nonoilseed sunflower and flax currently produced anywhere (Wolnik et al., 1983,

Table 4. Correlation (r) between kernel Cd and seedling leaf Cd level, and kernel Cd and diagnostic leaf Cd concentration at each location over 200 sunflower lines grown at 4 locations in North Dakota and Minnesota

Leaf Cd	Kernel Cd			
	Fargo	Crookston	Grandin	Breckenridge
Seedling leaf Cd	0.12	- 0.0063	0.14	0.12
Diagnostic leaf Cd	0.49***	0.46***	0.58***	0.36***

*** Significant at the 0.001 probability level.

1985; Ocker et al., 1991; Chaney et al., 1993). Sunflower and flax are minor foods. If the proposed 0.1 mg Cd/kg limit is established at CODEX, however, much more intensive research and breeding will be needed to identify multiple genetic sources of lower grain Cd in non-durum wheat, rice (*Oryza sativa* L.), corn (*Zea mays* L.) and soybean. Further, cultivar evaluation for grain Cd will be needed for both existing and all new commercial cultivars, and analysis of grain Cd is expensive. Plant breeders should be aware of this potential new breeding requirement, and of successful approaches to screen for lower Cd grain.

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